STUDIES IN ATYPICAL FORMS OF TUBERCLE BACILLI
ISOLATED DIRECTLY FROM THE HUMAN TISSUES
IN CASES OF PRIMARY CERVICAL ADENITIS.

WITH SPECIAL REFERENCE TO THE THEOBALD SMITH GLYCERINE
BOUILLON REACTION.

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PLATES XIV AND XV.

INTRODUCTION.

Koch's statement in 1901 (1) that bovine tubercle bacilli are quite incapable of infecting human beings has led to much careful research being devoted to the identification of the type of tubercle bacilli which can be derived from tuberculous lesions in man. As one of the criteria of type the glycerine-bouillon test introduced by Theobald Smith (2) in 1905 has been used to separate the human from the bovine type of the bacillus. It has now been established that the bovine bacillus can be implanted upon and grown in human tissues where they produce lesions of tuberculosis. My own studies have been devoted to the identification of the types of tubercle bacilli derived from cultures from a small series of cases of primary cervical adenitis. The clinical course of the disease in the several instances was quite similar while the tubercle bacilli isolated and studied carefully over a period of two years have proven to be distinct from one another. On the other hand, the clinical appearances observed in these cases were quite different from those usually observed in tuberculous cervical adenitis and led directly to my undertaking a study of the bacilli yielded by the lesions.

The four cultures which form the basis of this communication

1 Paper read before the International Tuberculosis Conference held at Washington, D. C., October, 1908. Received for publication November 14, 1908.
were studied with the view of determining, if possible; (1) the type of infection; (2) whether the growth features, reaction changes in glycerine bouillon, and virulence for rabbits and guinea-pigs, remain relatively constant for a period extending over years; (3) if transitory changes occur in virulence, growth and reaction in bouillon, which might explain the so-called "intermediate" types of bovine and human cultures which have been described; (4) whether the T. Smith glycerine-bouillon test serves to determine degrees of adaptation in man to bacilli of the lower host-species.

A pure culture was obtained in each case by transplanting portions of the infected tissues from the neck to a modified egg medium compounded and prepared as follows:

The yolks are carefully separated from the whites of twelve eggs and each dropped separately and aseptically into a flask containing 100 c.c. of human blood serum, 200 c.c. of distilled water and 10 c.c. of glycerine which has already been sterilized by the intermittent method of sterilization (Arnold). The flask is now thoroughly shaken and the mixture carefully tubed. These tubes are heated in the slanted position at a temperature not exceeding 70° C. until the consistence of the medium is sufficiently firm to insure its holding together when the tubes are in the upright posture. It is essential to obtain the egg mixture sterile in the test tubes before they are inspissated so that the one heating, which is necessary to solidify the medium, suffices. This gives a soft golden yellow medium with plenty of water of condensation. The cotton plugs are paraffined to keep the medium moist.

From the original cultures a growth upon glycerine bouillon was initiated by depositing upon sterile paper "floats" masses of egg culture. Further, guinea-pigs were inoculated subcutaneously with tuberculous material from the neck. After the tuberculous process was set up portions of the caseous glands were planted upon the modified egg medium. The growth thus obtained was transferred to glycerine bouillon by the method described above.

After the initial isolation, observations upon growth features, reaction changes in glycerine bouillon, and virulence for rabbits and guinea-pigs were observed as to their relative constancy over an extended period.

The glycerine bouillon tests were carried out with the following technique: three to six siphon-flasks were simultaneously inoculated from the same culture and controlled by similar transplants of human, bovine and avian standard cultures of the same age and grown upon the same medium.
Titrations were begun from the new cultures and the controls as soon as peripheral growth appeared about the floating transplants. It should be noted that before inoculation all the bouillon, which was prepared in the usual way from fresh muscle infusion, was accurately standardized and the percentage of acidity of each carefully tabulated. Throughout the experiments 500 c.c. flasks were employed in order to provide for the waste by titration and loss by evaporation and to make possible an accurate comparison of the inhibitive influence of the growth products of the different cultures. Titrations were repeated at intervals of one to two weeks over a period of six to twelve months. The quantity for all titration tests was 5 c.c. of the bouillon culture medium in 45 c.c. of distilled water. The initial acidity of the glycerine bouillon was varied, and a series of 1, 1.5, 2, 2.5 and 3 per cent. acidity was observed.

The virulence of the new cultures with relation to growth variations was determined from time to time and compared with the virulence of the standard type cultures by subcutaneous and intravenous inoculations of rabbits and guinea-pigs. The dosage was standardized as far as possible by weighing and emulsifying in physiological salt solution equal quantities of growth material from the various cultures.

THE CLINICAL CASES,

In all the cases the tuberculous process manifested itself as a rapidly increasing swelling of the neck, which in every way simulated an acute pyogenic infection. It was necessary in two of the patients to perform a tracheotomy to relieve the respiratory distress. A careful physical examination failed to show any signs elsewhere in the body of tuberculosis.

Three of the cases died of acute disseminated miliary tuberculosis in a few weeks after the first symptoms of cervical enlargement was noted. The fourth case was treated with tuberculin and apparently made a complete recovery.

The three autopsies showed a primary tubercular focus in the cervical lymph glands from which resulted acute general miliary tuberculosis.

The following are brief clinical notes and abstracts from the autopsy protocols:
Case I. Clinical History.—Mrs. L., 49 years old, a native of Canada, who lived on a farm all her life, entered the Montreal General Hospital June 4, 1906. Three weeks before admission she noticed for the first time a swelling on the left side of the neck, which rapidly increased in size during the next five days when it ruptured and discharged. In two weeks the swelling had spread over the entire left side of the neck from the lower jaw to the clavicle. At no time did the mass cause pain or give rise to tenderness on pressure.

Following incision a stained smear preparation of the pus was crowded with acid-fast bacilli. The bacilli occurred for the most part in closely aggregated masses, and in order to obtain them sufficiently scattered for a microscopic study it was necessary to dilute one part of the pus in three parts of water. The thinner parts of the expressed material contained myriads of pin-head and smaller sized reddish-brown granules which when crushed and stained proved to be pure masses of the bacilli.

A small quantity of the pus was transplanted upon modified egg-medium and also upon the ordinary culture media and incubated at 37° C. The egg mixture after four or five days became thickly studded with discrete colonies 1 mm. in size which proved to be acid-fast bacilli. All the inoculated tubes of ordinary media remained sterile, a fact especially significant in view of the necrotic condition of the tissues and the presence of an open avenue for secondary infection.

Full grown guinea-pigs and rabbits were inoculated subcutaneously and intraperitoneally with 0.5 c.c. each of the pus from the neck. The guinea-pig and rabbit that received intraperitoneal injections died within fifteen days of generalized tuberculosis. The rabbit inoculated subcutaneously died on the twenty-sixth day. The autopsy revealed an advanced tuberculosis; the lungs, liver and spleen showing the most extensive involvement. The guinea-pig similarly inoculated died on the fourteenth day of disseminated tuberculosis. Pure cultures of tubercle bacilli were recovered directly upon modified egg-medium from the spleen of each animal.

From the date of admission the patient failed rapidly and died five days later from acute general miliary tuberculosis; or four weeks after the first appearance of the disease.

Autopsy.—Sixteen hours post-mortem. Body of a well nourished woman. The whole left side of the neck is occupied by a large nodular mass which extends from the lower jaw to the clavicle and from the trapezius muscle to the median line in front. Over the tumor are three linear incisions.

The peritoneum and plurae are beset with disseminated miliary tubercles.

The lungs are crepitate throughout and everywhere beset with innumerable discrete tubercles. No evidence of old tuberculous foci.

The other organs aside from acute miliary tubercles show nothing remarkable.

A pure culture of B. tuberculosis was isolated from the heart's blood on egg-medium; twelve or more colonies developing from six platinum loopfuls of the blood.

Anatomical Diagnosis.—Primary tuberculosis of the right cervical lymph glands; acute general miliary tuberculosis; B. tuberculosis septicemia.

The tuberculosis in this case was undoubtedly primary in the
cervical glands, for a most careful search at autopsy failed to reveal any older focus.

In the human tissue the tubercle bacilli were quite long and slender and transversely segmented. They retained Gram's stain readily. Indeed, even in tissue-sections fixed in Zenker's fluid, embedded in paraffin and treated in the usual routine manner with eosin-methylene-blue the bacilli were stained intensely with eosin.

The bacilli were so numerous in the sections of the human spleen and lymph nodes that it was difficult to discern clearly the nuclei of the tissue cells even in fields where there was no necrosis. The organisms occurred without definite arrangement. Frequently the bacilli were packed so closely together that whole fields of the microscope consisted of one dense layer of slender, beaded rods (see Fig. 1, Plate XIV). In those parts of the sections where the bacilli were less dense, large numbers of them appeared within mononuclear leucocytes. These engorged cells were analogous to the phagocytes for red blood cells, so common in the lymphoid structures in typhoid fever. One could frequently count fifty or more bacilli within a single cell. The descriptions of occurrence of enormous numbers of leprosy bacilli in human tissues are inadequate to convey a correct idea of the picture here presented in tuberculosis. Lydia Rabino-witsch (3), however, describes a similar distribution of bacilli and phagocytic cells in tuberculous tissues of fowls.

The bacilli when cultivated on artificial medium presented an entire change in morphology from that observed in the tissues; instead of the long slender beaded rods appeared small plump bacilli almost coccal in character, and without a suggestion of transverse massing of the chromatin. On egg-medium the first generation presented a most curious arrangement of the bacilli, which even the manipulations with the platinum loop failed to disturb. The individual rods occurred one behind the other in parallel rows forming broad wavy bands that extended across many fields of the microscope. This end-to-end arrangement of the individual organisms is most unusual in tubercle bacilli and resembles the appearance met with in anthrax colonies.

The character of the growth on ordinary media employed for the cultivation of tubercle bacilli completely corresponds with the
human type of culture. The growth was always very dry. Though sometimes fine and film-like the culture at other times was luxuriant, lumpy and of wax-like whiteness. It never presented the "moist appearance" that is said to be characteristic of avian cultures. A feature of the bacillus was its prolonged viability on artificial media, a profuse growth having been obtained from the original culture, which had been sealed and kept corked at room temperature for more than two years. Lydia Rabinowitsch (3) claims that prolonged viability is a distinguishing feature of the avian bacillus; in some instances under refrigeration the culture remaining alive for two years. Culture I not only remained viable for a period of years at ordinary temperature but retained its virulence for rabbits and guinea-pigs.

The results of animal inoculation with Culture I afforded a complete illustration of the possible variation in the histological lesion. There was a distinct difference between the microscopic character of the human lesion and that produced experimentally. The subacute inflammatory type of lesion in man, which also occurred in the earlier experimental animals, was a striking feature, and would indicate an extremely high degree of virulence. The lesion in man consisted chiefly of lymphoid and plasma cells and large numbers of polymorphonuclear leucocytes, with only an occasional epithelioid type of cell and no giant cells. In the experimental lesion produced by the culture somewhat attenuated in virulence the epithelioid elements with central necrosis and giant cells predominated. Giant cells did not occur in the earlier rabbits and guinea-pigs inoculated, nor in the lesions of the chickens. In later experiments, however, the animal tissues showed numbers of giant cells associated with the lesions. It would seem that the occurrence of giant cells in tuberculosis, as in glanders (4), depends more on the virulence of the organism than on any other factor. This culture at least suggests that the type of lesion, whether exudative or proliferative, is due to the number and virulence of the bacilli.

Lydia Rabinowitsch (3) noted in avian tuberculosis, distinctive differences in the histological picture. In the fowl the alveoli of the lungs were filled with an exudate composed of epithelioid and lymphoid cells, together with numerous giant cells containing large numbers of the tubercle bacilli. This author attaches
special significance to these large cells filled with an enormous number of bacilli and regards them as characteristic of avian tuberculosis. This same histological picture is seen in the human and experimental lesions produced by Culture I. Here the lymphoid and large mononuclear cells were crowded with bacilli and presented an analogous picture in this respect to leprosy.

The culture, after it had become attenuated in virulence, produced, after repeated intravenous injection, a lesion in the glands of the rabbit which exhibited a certain histological resemblance to that of Hodgkin's disease such as Dorothy Reed (5), Longcope (6) and others have described.

The virulence of the culture was tested for rabbits and guinea-pigs, the animals being approximately of the same age and weight. Chickens, pigeons and rats were also used. Each comparative test was made intravenously, intraperitoneally and subcutaneously with rabbits, guinea-pigs and pullets. One milligramme of culture given intraperitoneally produced a fatal tuberculosis in these animals in four to six weeks. The same dose administered subcutaneously required a much longer time to produce the same result and in some instances both guinea-pigs and rabbits lived for months. Ten milligrammes, however, when injected subcutaneously, gave rise to disseminated tuberculosis in six to ten weeks.

A series of six chickens, three hens and three pullets, received each one milligramme of the culture intraperitoneally, subcutaneously and intravenously. Six weeks later the fowls were killed and the two pullets which had received intraperitoneal and intravenous injections showed well-marked lesions of tuberculosis. The mesentry of the intraperitoneally injected pullet presented numerous discrete 2 mm. sized tubereles (see Fig. 4, Plate XV). No lesions in the other organs were noted. The pullet which received an intravenous injection showed a number of small tubercular nodules in the lungs and spleen. The subcutaneously inoculated pullet and the three hens presented no trace of tuberculosis. When we consider the small dosage and the short period allotted the chickens to live the occurrence of macroscopic lesions is most remarkable. Larger doses invariably produced lesions in the half-grown chickens which as Bang (7) claims are far more susceptible than full-grown fowls. Positive results occurred in chickens following intraperitoneal inocu-
Chart 1. Showing the reaction upon glycerine broth of Culture 1 over continuous period of 30 months. Dots upon arrowed lines indicate titration intervals. Note the complete change in reaction for the fourteenth generation.
Chapter II. Illustrates the glycerine bouillon curves. Unbroken lines represent "Anypical reaction" curves.
The reaction curve for this culture in glycerine bouillon was decidedly atypical for more than two years. It always gave an increase in the percentage of acidity without the initial fall that is so characteristic of all tubercle cultures. The reaction was occasionally delayed for weeks after the growth had completely covered the bouillon surface (a feature in my experience not peculiar to any strain), but when the change in reaction began there always occurred a constant rise until an elevation of five or more points higher than the initial acidity was attained, after which there was no further change (see chart, Culture I).

For a period of more than two years after the culture was recovered from the human tissue the behavior towards glycerine broth was certainly unlike any known human or bovine type of organism since invariably it failed to produce the "initial fall" in the acidity percentage. Again during this period the culture did not behave in respect to glycerine bouillon as the control avian culture (Smith 2) which as O. Bang (8) first pointed out, describes the same reaction curve as the bovine, namely, a gradual decline in percentage acidity until an alkaline reaction results. Quite recently, however, this culture has given a complete change in the reaction curve.

It has been my practice each month since the isolation of the culture to make fresh transplants upon glycerinated bouillon to determine whether subsequent generations might not show a variation in reaction. I was therefore surprised to discover after two and one-half years that the culture suddenly underwent a complete reversal in its action upon glycerine producing now the typical curve of the avian bacilli (see Chart I).

The organism from this case was regarded at first as a modified avian bacillus or an "intermediate" between the avian and human types. Its prolonged viability under artificial conditions and the production of tuberculosis in the fowl are points indicating an avian histogenesis, but the former high acid production and the absence of moist growth on media do not correspond to the typical avian bacillus. However, the recent behavior upon glycerine bouillon leaves little doubt that it is avian in type.
CASE II. Clinical History.—Samuel B., 20 years old, a native of Ireland, entered the Montreal General Hospital in October, 1906, complaining of shortness of breath and great weakness. Two weeks before admission he had noticed for the first time a swelling on the right side of the neck, which rapidly increased until it attained approximately the size of an orange.

The swelling was opened under surgical precautions and a large amount of creamy yellow material removed. A portion of this was immediately spread over modified egg medium which after three weeks at 37° C. developed a pure culture of the tubercle bacillus. The ordinary culture media inoculated at the same time remained sterile. Stained smear preparations of the pus from the neck showed only a moderate number of short, thin, acid-fast bacilli. The first growth on artificial media was not luxuriant and rather slow in development, but there was no difficulty in getting transplanted “floats” from the original culture to grow on glycerine bouillon. Pus was injected intraperitoneally and subcutaneously into rabbits and guinea-pigs. The rabbits which received an intraperitoneal inoculation died six weeks later of generalized tuberculosis, while the one which had been injected subcutaneously, when killed four months afterwards, showed only tuberculosis about the site of inoculation and small nodules in the lungs. Both guinea-pigs died of tuberculosis; the intraperitoneal animal in five weeks and the subcutaneous one in nine weeks.

Autopsy.—Fifteen hours after death. Subject emaciated. On the right side of the neck there is a swelling that extends from the jaw downward and behind the clavicle. Over this area is an incision 3 cm. in length which exposes a large mass of yellowish-white necrotic material. On opening the mass it is found to be continuous with another in the anterior mediastinum which is approximately 12 cm. by 16 cm. in extent and completely encircles the trachea. The other superficial lymphatic glands of the body are not enlarged.

All the organs contain miliary tubercles.

Anatomical Diagnosis.—Glandular tuberculosis; acute miliary tuberculosis of the liver, spleen and kidneys; acute tuberculous pleuritis.

The organism recovered from this case—designated Culture II—belongs undoubtedly to the human group of tubercle bacilli. Though the growth was always slow to develop on glycerine bouillon the culture corresponds in other features, especially virulence for rabbits, to the human variety—and further, while the bio-chemical reaction as interpreted by glycerine bouillon titrations showed at times aberrations from the normal, the curve furnished by this organism was found on the whole to tally closely with the human type.

CASE III. Clinical History.—Francesco B., 24 years old, entered the Montreal General Hospital in October, 1906, complaining of a swollen neck and difficulty in breathing and swallowing. Personal history negative. There were fluctuating masses on both sides of the neck extending from the mastoid processes to the clavicles.

The third day after admission the mass on the right side of the neck was incised and 16 ounces of thick creamy pus and caseous material removed. The
mass on the left side was opened on the following day and 8 ounces of pus evacuated. A portion of the pus was transferred to egg medium which showed a pure growth of tubercle bacilli after ten days' incubation.

A pair of guinea-pigs and rabbits were inoculated subcutaneously and intraperitoneally with a portion of an emulsion prepared from the discharge. The rabbits were killed, one four weeks, the other three months after inoculation. The intraperitoneally injected animal showed no trace of tuberculosis while the one which was subcutaneously injected contained definite tuberculous lesions at the site of injection and in the glands of the groin. The guinea-pigs injected had increased in weight. The superficial inguinal glands of the subcutaneously inoculated animal were enlarged and contained infarcts and caseous nodules. In the intraperitoneally inoculated pig the only tubercular lesion presented itself in the omentum. The cultures recovered from these animals in no way differed from the one obtained directly upon artificial medium from the human tissues.

The patient died on October 31 of sudden cardiac failure, five weeks after the first symptoms were noted.

Autopsy.—Thirteen hours post-mortem. On either side of the neck, which is enormously swollen, there is a deep incision 3 cm. in length, the edges of which are gangrenous.

Peritoneal Cavity.—The omentum and intestines are glued together by recent fibrinous adhesions. The parietal peritoneum is studded with glistening tubercles. The mesenteric lymph nodes are enlarged and caseous.

The lungs, spleen, liver, kidneys and brain are thickly studded with minute pin-point translucent tubercles. No old lesion in the lungs.

Cecum, descending part of the colon and the lower part of the ileum contain numerous tuberculous ulcers.

Anatomical Diagnosis.—Primary tuberculosis of the cervical glands; acute general miliary tuberculosis; acute ulcerative enterocolitis.

A comparative study of the culture isolated from this case—designated Culture III (atypical)—corresponds with the human variety of tubercle except in regard to its behavior at times upon glycerine broth where it yields a neutral or 0.5 per cent. alkaline reaction before the rise in acidity is established.

The culture possesses a low degree of virulence for guinea-pigs and rabbits. Full-grown rabbits seldom developed tuberculosis following intraperitoneal injections of ten milligrammes of fresh culture. Adult guinea-pigs inoculated intraperitoneally with the same amount frequently increased in weight and when killed three months afterwards showed only a few nodules in the omentum.

Case IV. Clinical History.—Antonio C., aged 16, entered the Montreal General Hospital in March, 1908, complaining of a swollen neck. The cervical glands are enormously enlarged on both sides of the neck and range in size from a marble to that of a hen's egg. They are discrete, freely movable and give no tenderness on pressure. The mediastinal glands on percussion are found greatly
enlarged and apparently continuous with the cervical mass. The axillary, inguinal and other superficial glands of the body are not enlarged, but the abdominal glands can be distinctly palpated. The heart and lungs are normal. One week after entrance to the wards the swelling of the neck greatly increased in size and became less nodular in character; instead of freely movable, discrete masses there is now a solid tumor which extends from the jaw downward under the clavicles and into the mediastinum. The swelling was opened under aseptic precautions and a gland 2 by 1 cm. in diameter removed for bacteriological study. On section it was firm and contained large yellowish-white areas; no bacilli, however, were demonstrable in the stained smears. Cultures were obtained directly upon egg medium from transplanted pieces which were excised from the central part of the gland. The ordinary laboratory media inoculated remained sterile. The egg medium showed the first sign of growth after three weeks when it became studded with small colonies of the tubercle bacillus.

Soon after admission the patient was given at regular intervals in gradually increasing doses T.R. (tuberculin), under which treatment the cervical enlargement completely subsided. The glandular mass in the neck began to diminish in size from the first dose. Those in the mediastinum and the abdomen also disappeared. The patient gained markedly in weight and the temperature fell to and remained normal. He was discharged apparently well, one month after the first administration of the tuberculin.

The culture from this case, designated IV, completely corresponds in respect to growth and reaction upon glycerine bouillon to the standard bovine type; however, it does not correspond in degree of virulence for rabbits. Fifty milligrammes of fresh culture emulsified in 5 c.c. of sterile salt solution and injected intravenously into the ear of the rabbit failed repeatedly to produce lesions. Rabbits inoculated subcutaneously with the same dosage did not develop disseminated tuberculosis; and when killed months afterwards presented lesions only at the site of inoculation or in the regional lymph glands. On the other hand, guinea-pigs injected intraperitoneally with ten milligrammes of the culture rapidly developed generalized tuberculosis. As yet I have not tested the virulence of the culture for calves. In respect to growth the culture always manifested a tendency to develop slowly. Though under artificial cultivation for nearly a year it still refuses to grow on glycerine agar. On glycerinated bouillon growth increases very slowly though always as a heavy colony-mass and at no time film-like which is so often the case in feebly growing bovine cultures. For the present, at least, I regard Culture IV as an atypical bovine variety of the tubercle bacillus possessing a low degree of virulence for rabbits. Atypical
cultures producing a neutral or slightly alkaline reaction upon glycerine broth have been described by Theobald Smith (9) and O. Bang (8) and regarded by them as “atypical” human rather than bovine in type; however, the slow multiplication of Culture IV on the more favorable media and the inability to grow at all on glycerine agar, together with the fact that it produces a neutral or slightly alkaline reaction are factors strongly in favor of a bovine nature.

THE GLYCERINE BOUILLON REACTION AS A DIFFERENTIAL TEST.

Theobald Smith (10) in 1905 described the difference in behavior of “bovine” and “human” bacilli towards glycerine bouillon which corresponded with the author's previously recognized variation in virulence. Before the glycerine broth test was known, however, the differentiation of these depended mainly on virulence and rapidity of growth. The feeble and slowly growing organism if highly virulent for rabbits when injected intravenously, was regarded as bovine in type; while the luxuriant and rapidly growing culture that possessed a low degree of virulence for rabbits was classed as the human variety.

In my experience the glycerine bouillon test is more reliable than “degree of virulence” in the differentiation of mammalian strains provided it is repeated over a long period of time. Even with regard to avian cultures O. Bang (8) claims that the alkalinity produced in glycerine broth is so much greater than with the bovine type that it serves to distinguish between them. My observations upon the behavior of Culture I are in accord with this finding.

The test, which consists of growing the organism on the surface of glycerine bouillon and titrating from time to time to note the change in reaction, not only divides the tubercle family into two distinct groups but it may be a qualitative means of detecting bacilli of the modified bovine type in the human body or the degree of adaptation. Though the method does not sharply differentiate between bacilli of the lower host-species it is of aid in the identification of these lower type forms in the human body. Difference in virulence and character of growth are too variable to allow us to admit unreservedly that one type may become transformed into another through animal passage. These properties even with the
same culture, vary within wide limits, more especially in the growth features which depend so largely on the nature of the culture medium. Yet it would seem that these modifications in biological and pathogenic characters have been the crucial tests employed by those who claim to have transformed the avian into the bovine type of bacillus.

L. Rabinowitsch (3) in a study of 95 avian cultures noted a whole growth scale from the typical moist shiny growth of the avian to the dry scaly appearance of the mammalian type of bacillus, and is most emphatic that from three birds she isolated cultures that were identical with the human type of bacillus. She also claims to have completely transformed a typical avian culture into the human type of bacillus by repeated passage through animals. In view of the fact that she draws particular attention to the inconstant and interchangeable differences in growth and virulence of the mammalian and avian bacilli and that in morphology and staining reaction there is no essential variation, it is hard to understand how "atypical" forms were definitely determined, especially as there is no mention of the glycerine bouillon reaction. If we regard the tubercle bacilli of the different host-species as variations of the same family the existence of "intermediate forms" is not only possible but highly probable. There is reason to believe that aberrant types of the tubercle bacillus exist as they do for other bacterial species, but there is as yet no absolute test by which the finer differences may be determined. Our present methods at best are too inadequate to permit us to state positively with regard to "intermediate" forms of the tubercle bacillus. Some so-called atypical cultures that have at first behaved unlike the standard types if repeatedly tested over a longer period of time might eventually fall into the well-known groups. The most striking examples of this change have occurred with Cultures I and III of this series.

It would seem that these strains have only recently accepted a new environment and are but slightly altered by the host influence. In this way it is possible to explain their odd behavior upon glycerine broth after a year's sojourn outside the animal body. For more than a year Culture III, though constantly under cultivation and repeatedly tested, always approached, but never reached, the neutral
TABLE I.

Showing the Reaction of Glycerine Bouillon for Cultures under Different Temperature Conditions.

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<tr>
<td>Beef Inf. Pepto c.c. 250 g, Glycine 45 c.c. Acidity 6.0 per cent.</td>
<td>Franc. II., age 24, native of Italy. Primary cervical adenitis resulting in fatal acute military tuberculosis.</td>
<td>Fus from cervical region. Direct recovery of culture from human tissue upon modified egg medium.</td>
<td>Moderately virulent for rabbits. Highly virulent for guinea-pigs. Avirulent for chickens.</td>
<td>Feb. 18, 1898</td>
<td>&quot; 25, &quot;</td>
<td>0.0 Neutral</td>
<td>Growth heavy and blistered and covers two-thirds of surface. Growth complete and up sides of flask.</td>
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<td>Sam. B., age 20, native of Ireland. Primary cervical adenitis resulting in fatal acute military tuberculosis.</td>
<td>III (Flask &quot;b&quot;).</td>
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<td>February 8, 1898</td>
<td>&quot; 25, &quot;</td>
<td>0.4 Slightly alkaline</td>
<td>Growth complete and up sides of flask.</td>
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<td>Beef Inf. Pepto c.c. 250 g, Glycine 45 c.c. Acidity 6.0 per cent.</td>
<td>Sam. B., age 20, native of Ireland. Primary cervical adenitis resulting in fatal acute military tuberculosis.</td>
<td>Necrotic material from the cervical glands. Direct isolation of culture from the human tissue upon modified egg medium.</td>
<td>Low degree virulence for rabbits. Virulent for guinea-pigs. Avirulent for chickens.</td>
<td>March 24, 1898</td>
<td>&quot; 20, &quot;</td>
<td>0.7</td>
<td>Growth over whole surface and up sides of flask.</td>
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<th>Incubation Date</th>
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point when grown in glycerine bouillon, nor was there any rise in acidity though the flasks were kept for months. Subsequent tests, however, have shown the fall in reaction to the neutral point to be followed by a rise in acidity. On two occasions the reaction fell slightly below the neutral point and later arose in acidity higher than the original percentage (see Table I). This latent action on glycerine occurred not only for the culture recovered directly from the human tissue but also for the one recovered through guinea-pigs. The question of a mixed tubercle infection is possible but hardly probable in this case. The glycerine broth test at first indicated that the culture was a "modified" bovine but its subsequent behavior in this medium together with other biological peculiarities make me regard it as an aberrant human type.

Culture I is the most significant example of the necessity for continued observation over a long period of time. For more than two years it always gave an atypical reaction curve in glycerine bouillon. The medium steadily increased in percentage acidity without fluctuation until it reached four or five points above the initial per cent. where it remained stationary. Recently, however, the culture has shown a complete change in its behavior upon glycerine broth; instead of the rise in percentage acidity there now occurs a gradual uninterrupted fall to 0.5 to 1.0 per cent. alkalinity from which there is no further change. This reversal of the metabolic activity of this culture under artificial cultivation is extremely interesting and would argue (1) a mixed infection in which the least saprophytic type at first predominated and subsequently the more saprophytic variety predominated, or (2) that the culture was originally an avian whose vital function had been altered during its sojourn in the animal body but not sufficiently to establish it permanently.

These "atypical" cultures in glycerine bouillon show on the whole a variation in reaction from the standard types analogous to the variation of the pneumococcus in inulin, which difference in degree of acidity corresponds with differences in growth and virulence.

Explanation of the Glycerine Bouillon Reaction.—To determine the effect of growth upon the glycerine bouillon reaction the following experiments were carried out: A series of glycerine bouillon
cultures of bovine, human and avian bacilli were grown for a stated period at 37° C. All the flasks were then titrated and the reactions carefully noted. Next, one-half of these cultures (a complete series) was retained at room temperature for a further period and observations upon the reactions carried out at intervals of five and ten days. Finally, the remaining half of the cultures after titration (also a complete series) was transferred from incubator to ice-chest temperature and the reaction noted from time to time under the latter conditions. Observations extended for some series over a period of six months, others three months, and in none was it less than eight weeks. Flasks holding 500 c.c. were employed in the tests in order to contain a sufficient amount of bouillon to permit of repeated removal for titration purposes. In these tests the amount of surface growth and the reaction of the bouillon was purposely varied. The surface growth was shaken to the bottom of the flasks upon removal from the incubator. Under these conditions it was interesting to note that the human type of tubercle showed not the slightest difference in the final reaction from cultures kept undisturbed at 37° C. In flasks containing the human strains where the reaction was beginning to fall toward the neutral point at the time of removal from the incubator the acidity did not continue to lower but eventually rose to a point equal to or higher than the initial acidity (see Table I). It made not the slightest difference in the final reaction whether the growth had been shaken down from the surface of the bouillon or not. Flasks containing the bovine and avian types showed no further change in either direction after they were removed from the incubator.

The result of this test does not support the view that the difference in reaction for human and bovine mammalian types of the tubercle family depends on the ability of the living bacilli to split the glycerine molecule with the production of acids, but suggests an autolytic action on the glycerine. Again, avian strains, according to O. Bang (8) who has tested their action upon glycerine bouillon, produce only a neutral or alkaline reaction when growing

8Bovine IX and VIII, human XII, and avian II, which were kindly supplied me by Prof. Theobald Smith; and Cultures I, II, III and IV of my own series.
Charles W. Duval.

on the surface of the medium, which fact also shows that neutralization of the initial acidity is the result of growth activity.

Sometimes with a series of glycerine bouillon cultures one or more of them may fail to show the slightest change in reaction for months though the growth is complete and heavy over the whole surface and growing up the sides of the flask. At times a flask whose growth was "complete and down" for more than two months and so far had not shown the slightest change in reaction would suddenly begin to rise in per cent. acidity (see Table I).

Occasionally the change in reaction may be so long delayed that the fall to the neutral points occurs before the rise in acidity begins. Again, it is well known that with all types when grown in a broth where no glycerine has been added the reaction drops to neutral or becomes slightly alkaline and at no time shows a rise in acidity. This trend towards the neutral point must take place in glycerinated bouillon as well; however, with the human type there is in addition an action on the glycerine molecule which is distinct, and at times sufficient not only to lower but to cause a rise in the acidity of the glycerine bouillon. Since the number of bacilli undergoing disintegration varies constantly this factor in itself determines the degree of fluctuation.

That autolysis explains the rise in acidity for the human type of tubercle bacillus when grown in glycerine broth the following experiments show. A series of glycerine bouillon flasks were first titrated to show distinct variations in reaction, viz., neutral 0.5, 1.0, 2.0, and 3.0 per cent. acidity. To the separate flasks were now added five grammes of a bovine, human and avian culture which had been previously grown and removed under sterile precautions from other bouillon flasks. Each flask was now titrated to note whether any change in the reaction had occurred immediately following the addition of the culture, and then placed in the oven at 56° C. for six hours to kill the bacilli. Again after heating, the reaction was determined and the flasks placed at ice-chest temperature. Up to this date no change in reaction for any flask had occurred. Four days later the flasks still showed no change from the original reaction. About the eighth day, however, the flasks containing the killed "human" bacilli showed a decided rise in percentage acidity.
which from day to day continued to advance until the final reaction
was equivalent to that attained by the living glycerine-bouillon-
culture under favorable conditions. The flasks containing the killed
"bovine" and "avian" bacilli, respectively, failed to show at any
time the slightest change in the reaction.

The fact that increased acidity occurred in the flasks containing
the dead "human" bacilli and not in the "bovine" or "avian"
would indicate that in the autolysis some soluble substance was
liberated from the human strain that had a special action upon the
glycerine molecule.

The above explanation in the rise in the acidity of glycerine cul-
tures of human tubercle bacilli is, of course, at variance with the
statement of the English Commission (11) that this phenomenon
was due to the chemical interaction of continued metabolic activity
on the part of the human strain.

VIRULENCE.

It is conceded by many that a considerable range in degree of
virulence is possessed by the human type of the tubercle bacillus, but
T. Smith (12) holds that the most virulent human cultures are not
to be compared in virulence with the bovine strains. On the other
hand, L. Rabinowitsch (13) states that some human cultures are
more virulent for rabbits than the bovine. In this connection it is
noteworthy that Culture IV of this series which is a typical bovine
type of bacillus according to growth and behavior towards glycerine
bouillon, has always manifested a low degree of virulence for
rabbits.

Culture I when first isolated was equally virulent for rabbits and
guinea-pigs and also virulent for chickens. Rabbits succumb ap-
proximately within the same period as do guinea-pigs following
intravenous injection of ten milligrammes of the culture.

The view held by Arloing (14) and his pupils that bacilli from
surgical tuberculosis are less virulent than those of pulmonary dis-
ease is not borne out by the study of these cultures which proves
that glandular tuberculosis is not always due to bacilli of reduced
virulence or to increased resistance of the host.

Again, Culture I is the exception to the rule that with tubercle
bacilli there is an inverse relation between the virulence and rapidity of growth. It grew profusely from the beginning and never showed the least tendency to lag when transferred from the solid medium to glycerine bouillon. Old cultures of the organism when kept at room temperature in sealed tubes for more than two years have remained highly virulent for guinea-pigs and rabbits; one milligramme of the culture producing extensive tuberculosis and death of the animals in four to six weeks.

These cases in some respects resemble clinically one reported by T. Smith (15) in 1898; though in his case there was an old lung focus to which he ascribed the subsequent cervical enlargement. He concluded that the rapid multiplication of the bacilli in the glands of the neck was probably due to the fortuitous assistance of other bacteria accidentally present or subsequently introduced. His culture, however, was a human type of tubercle possessing a low degree of virulence.

**HISTOPATHOLOGICAL LESIONS.**

Both in regard to the human and experimental lesions produced by the cultures herein reported there occurred distinct differences in the microscopic appearance, but in no way could these differences be considered as specific for any particular strain. All gradations from a subacute exudative to a proliferative type of lesion was observed.

The histopathological differences as described by Pfander (16) for fowl tuberculosis, such as (1) a relative scarcity of giant cells (2) caseation that is smooth, solid and hyaline with little tendency to calcify (3) and a tendency of the tubercle to become surrounded by a connective tissue capsule, I have observed but found too inconsistent to be of any differential value. Again, I have not been able to confirm Baumgarten's (17) view that the tubercles in fowls have less leucocytic infiltration than those in mammals. L. Rabinowitsch (3) states that in chicken tuberculosis there is a tendency for the bacilli to be diffusely disseminated and to occur in definite "heaps" which she has never observed in lesions of experimental mammalian cultures and she regards these differences as peculiar to birds, though not dependent upon any one type of tubercle bacillus. This "heaping" up of the bacilli and their spreading to all
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parts of the animal body is beautifully demonstrated in Case I, which would suggest an avian infection in man.

In general the experimental lesions fall under three types: (1) the subacute exudative which is composed of polymorphonuclear leucocytes, lymphoid and plasma cells without giant or epithelioid cells; (2) one that consists entirely of epithelioid cells without necrosis or exudation, a type of lesion similar to the proliferated endothelium of the Malpighian bodies of the spleen in diphtheria and other acute infectious diseases; (3) the typical tubercular lesion consisting of epithelioid, giant cells and necrosis. All three types of lesion with intermediate stages can be produced with the same culture by grading the dosage according to the virulence and number of bacilli.

SUMMARY.

The four cultures which form the basis of this communication were recovered from peculiar cases of primary cervical adenitis in man, three of which terminated fatally of disseminated acute miliary tuberculosis in four to six weeks.

A careful comparative study shows that Culture II corresponds closely with the “human” and Culture IV with the “bovine” type of tubercle bacilli; while Cultures I and III present variations from the standard types and are to be regarded as “intermediate” or “atypical” forms.

Culture I is of unusual interest because of its remarkable variations. The clinical picture of the case, the rapid course of the infection, the enormous number of the bacilli in the tissues, their tendency to occur in “heaps” like the leprosy bacillus, the high degree of virulence alike for rabbits and guinea-pigs, the production of lesions in chickens, the ease of cultivation and the prolonged viability under unfavorable conditions, all mark the organism as a decided atypical form of tubercle bacillus in man. The prolonged viability, the production of lesions in the chicken and the great profusion of bacillary growth in the tissues would indicate an avian type. Though for years the reaction curve was atypical it has since changed completely to the “avian” curve. In this connection it is of interest to note that L. Rabinowitsch (3) states that she has iso-
lated avian tubercle bacilli from two cases of tuberculosis in man.

Cultures II and III undoubtedly belong to the human type of the tubercle family though they were under cultivation and were repeatedly tested upon glycerine broth over a period of months before their identity was definitely established.

Culture IV completely corresponds in growth and reaction in glycerine bouillon to the bovine strain; however, it manifests a low degree of virulence for rabbits which is exceptional for bovine cultures.

The old belief that bovine bacilli are more slender and beaded in the tissues and are thicker and shorter in culture than the human type, I have not been able to confirm. The morphological characters of the different cultures here reported were so inconstant that no reliance could be placed on this feature as an aid in differentiation. Outside of the animal body it would seem that the differences in size and character of the individual bacilli depend largely on the kind and reaction of the medium, whilst in the animal body they are influenced by their situation and the resistance of the host.

The nature of the growth of these tubercle cultures varies for the same culture even under apparently identical conditions. The character of the growth was never an indication of the type of the culture. It was common to obtain two distinct types of growth on the same flask of bouillon, i. e., a portion of the surface would be covered with a heavy and uniformly granular layer of closely packed wax-like colonies twice the size of an ordinary pin's head, while the other portion would be a dense homogeneous layer with the typical depressed blisters.

The rapidity of growth also varied greatly for the same culture. Often in a series of twelve or more bouillon flasks which were prepared alike and inoculated with the same culture, some would cover the surface in eight days to two weeks, others would take four to six weeks, still others two to three months. It was thought in the beginning of the work that this variation might depend on the amount of oxygen within the flask or on the change in reaction in the bouillon, but further tests proved that neither of these influenced the rate of growth in any way. It would occur in loosely corked flasks as well as in those that were sealed, and in flasks where the
reaction was neutral, acid or slightly alkaline. It would seem that these changes are by no means specific for any group of the tubercle bacilli but a property possessed by them all.

The growth of the cultures on solid medium showed approximately the same variation as that from the surface of the glycerine bouillon. The wax-like colonies described by L. Rabinowitsch (3) as characteristic for avian tubercle bacilli were noted at times for all of the cultures. On the modified egg mixture the growth was always more rapid and profuse than on any other medium. I found this egg medium more certain than any other for the direct recovery of the tubercle bacillus from the tissues. Where it was desired to recover the culture from the animal tissues with certainty and celerity it had no equal. Occasionally in seven days after the inoculation of the tuberculous gland material the growth was sufficiently advanced to transplant to the bouillon flasks.

The glycerine bouillon test serves admirably to distinguish between the human, bovine and avian types of tubercle bacilli. It is also of value in the determination of degrees of adaptation in man for bacilli of the lower host-species, and in the recognition of "intermediate" types. The test to be of differential value requires repeated application and careful control over a period of months. Some freshly isolated cultures may produce their specific reaction curve in glycerine bouillon within a few weeks. On the other hand the same culture may fail to give its characteristic reaction or any alteration in the glycerine bouillon for several months though the growth has been luxuriant and complete.

The rise in acidity that occurs in glycerine bouillon for the human type of tubercle bacilli is due to a specific action on the glycerine of the products of disintegration of bacilli (autolysis); with the bovine and avian types the products of bacillary disintegration have no action on the glycerine. The fall in acidity which occurs for all three types of the tubercle bacillus is due to the products of metabolic activity.
Fig. 1.
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5. *Johns Hopkins Hospital Reports*, 1902, x, 133.
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EXPLANATION OF PLATES XIV AND XV.

Fig. 1. Colored drawing from a section of the human spleen (Case I.) to illustrate the enormous numbers of acid-fast bacilli in the tissues. The section is stained in carbol-fuchsin, decolorized in Orth's discharging fluid and then treated with a modified Romanowski stain (Duval).

Fig. 2. Section of a lymph node of the rabbit showing the histological changes that occur after repeated intravenous injections of Culture I. Note the similarity of the cellular picture to Hodgkin's disease.

Fig. 3–5. Illustrate the lesions in the chicken following intravenous and intraperitoneal inoculations of Culture I. (a) Isolated tubercle in the omentum; (b) Multiple tubercles in the mesentery; (c) Large caseating nodule.

Fig. 6. Sections from the lung of the rabbit inoculated intravenously with Culture I. Note the solitary tubercle composed of epithelioid cells and surrounded with a well-defined zone of lymphoid cells.